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POSTER ABSTRACTS

Poster session:
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11:30-12:30

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POSTER 1 NAM informed read-across of 2-Ethylbutyric acid repeat-dose toxicity from other branched carboxylic acids

Ciarán Fisher[1] and Sylvia Escher[2] on behalf of the Case Study 1 team.

[1] *Certara UK Limited, Simcyp Division, United Kingdom*
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Abstract:

In this proof of concept scenairo, 2-Ethylbutyric acid (2-EBA) was treated as a novel compound produced within the EU at over 100 tonnes per annum and so requiring registration under REACH, necessitating a 90-day oral exposure study. Specifically, to assess the hepatotoxic potential of 2-EBA, given the adverse findings identified for analogus compounds (2-Ethylheanoic acid, Valproic acid) in subacute/subchronic oral exposure studies; in particular hepatic steatosis. A category read-across (RAX) approach (scenairo 4) was adopted to obviate the need for a new in vivo study based on the in vivo data for analogus compounds and new approach methodologies (NAMs). Based on an adverse outcome pathway (AOP) describing the pathogenesis of hepatic steatosis, toxicodynamics was assessed through a battery of in vitro assays identified to quantify mutiple molecular initiating events (MIEs) and key events (KEs) in the AOP. The concentration range selected for testing in the in vitro test battery was informed by a reverse-translation physiologically-based kinetic (RT-PBK) modelling approach. RT-PBK modelling and simualtion predicted the hepatic concentrations achieved in vivo for rat VPA oral dosing studies identiying hepatic steaosis as a concern. Toxicokinetics across the category were predicted using an in vitro to in vivo extrpolation coupled PBK (IVIVE-PBK) approach, where PBK models are parameterised based on data generated in vitro (e.g. plasma protein binding, intrinisc hepatic clearance). This RAX integration of in vitro and in silico NAMs demonstrated consistent trends across the category with respect to toxicokinetics and toxicodynamics, concluding that 2-EBA will not result in hepatic steatosis in vivo.

POSTER 2 Case study 1: Learnings from OECD submission

Sylvia E. Escher[1], the Case Study 1 team, and the Working Group Core Team

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Abstract:

The EU-ToxRisk project aims to promote the regulatory acceptance of mechanistic risk assessment approaches, which are based on data from relevant in vitro and in silico models. Based on the learnings of the EU-ToxRisk read-across case studies a read-across concept1 was recently published, which illustrates schematically the integrating of NAMs data. The OECD case study project "Integrated Approaches to Testing and Assessment (IATA)" reviewed four RAX case studies, including case study 1. This poster will outline the main findings/comments from the different OECD countries. It turned out, that the opinions and recommendations differ within the assessment group.

A few highlights: the majority of the reviewers highly appreciated the application of an AOP network, and the systematic integration of all in vitro data based on it. Also, the use of a negative compound was considered to help within the assessment. The use of PBPBK modelling was received positively, although some reviewers asked for more guidance on how to use and interprete such complex models. The structure of the reporting template was not always considered to be appropriate, a finding that is also shared within the case study 1 team. To achieve a better understanding on the different pieces of information and how they support the hazard assessment, the published RAX-workflow was added to the document.

At the 5th Meeting of the IATA Case Studies Project under the remit of the Working Party on Hazard Assessment, the discussion focused on the uncertainty associated with AOPs that did not undergo a review by the OECD. It was, however, noted that also non-endorsed AOPs might be used for hazard characterization, as long as clear reference is made to peer-reviewed publications. Further, the rationale for selecting certain MIEs and KE, while leaving out others, has to be made clear in the RAX strategy and its documentation. Testing of every single MIE and KE along the network was not considered necessary in a read-across context and the proposed targeted testing was approved. The case study was accepted for publication by OECD in 2020.

POSTER 3 Systematic assessment of cellular gene network modulation by valproic acid analogues for biological read-across

Matthias Wehr[1], Nanette G. Vrijenhoek[2], Annette Bitsch[1], Liliana Capinha[3], Paul Walker[4], Paul Jennings[3], Florian Caiment[5], Laia Tolosa Pardo[6], Bob van de Water[2], Sylvia E. Escher[1]

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Abstract:

The read-across (RAx) compounds for this study have been selected based on their structural similarity and supporting in vivo information on shared toxicodynamic and kinetic properties. The selected of compounds include known liver steatotic and non-steatotic carboxylic acid which are to be grouped based on their gene expression profiles.

For this a transcriptomics analysis based on the TempO-Seq assay and the Human S1500+ Platform has been carried out on six concentrations per compound. These assays have been repeated for different liver cell types starting with the HepG2 cells and advancing to metabolically more active models like 3D-HepaRGs and primary human hepatocytes. Further RPTEC/TERT1 kidney cell are analysed for target organ comparison. We carried out a transcriptomics analysis to find differentially expressed genes (DEGs) per condition of treatment and to quantitatively compare between cell types. Furthermore, we carried out gene set analysis based on these DEGs for a qualitative comparison and mode of action analysis within RAX-compounds.

Initial results show a concentration dependent response in terms of number of DEGs for active compounds and a less pronounced response for the inactive compounds. There is some overlap of DEGs between the active compounds which enables us to cluster active/inactive compounds using the fold changes of the identified DEGs. The qualitative analysis shows a relation of these genes with several related pathways including e.g. genesis of adipocytes.

POSTER 4 Read-across based filling of developmental toxicity data gap for methyl hexanoic acid

Dinant Kroese[1], Tanja Waldmann[2], Jaffar Kisitu[2], Jonathan Blum[2], Katharina Brotzmann[3], Claudia McGinnis[4], Manuela Jaskin[4], Bjorn Koch[5], Barbara van Vugt-Lussenburg[6], Bart van der Burg[6], Ciaran Fischer[7], Iain Gardner[7], Oliver Hatley[7], Ségolène Siméon[8], Frederic Bois[7], Tony Long[9], Ulf Norinder[10], Barbara Zdrazil[11], Andre Wolterbeek[1]

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Abstract:

Read-across is one of the most often applied alternative tools for hazard assessment, in particular for complex endpoints such as toxicity after repeated exposure or developmental and reproductive toxicity. We have applied this approach in a case study to a series of six aliphatic carboxylic acids that have developmental toxicity data, some being positive, some negative. For one of these compounds, 2-Methylhexanoic acid (MHA), we have specifically blinded this toxicity data, and applied new approach methodologies (NAM) to substantiate the read across of the other compounds (as source compounds) to MHA, and to explore whether these NAM correctly predict the in vivo developmental toxicity of MHA. NAM applied were the Zebrafish Embryo Test (ZET), mouse Embryonic Stem cell Test (mEST), iPSC-based neurodevelopmental model (UKN1), and a series of CALUX Reporter assays, tests with clear relevance to DART, and these were combined with toxicokinetic models to calculate effective target concentrations and associated in vivo exposure doses. The NAM quite well predicted the in vivo developmental outcome of these aliphatic carboxylic acids, though for specifically MHA, being in vivo negative, this could not be confirmed with full certainty.

POSTER 5 Learnings from regulatory feedback on methyl hexanoic acid read across case study

Dinant Kroese[1], Tanja Waldmann[2], Jaffar Kisitu[2], Barbara van Vugt-Lussenburg[3], Bart van der Burg[3], Ciaran Fischer[4]

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Abstract:

The read-across case study on filling the developmental toxicity data gap for methyl hexanoic acid, 'EU-ToxRisk Case Study 2' was reviewed by the projects Regulatory Advisory Board, and by members of the OECD IATA Case studies project. Main comments were on endpoint coverage claim (developmental toxicity instead of DART; what is exact information requirement waived), validity of in vivo data used (Nau protocol is no full OECD 414), choice of target chemical (why not selected MPA, truly missing in vivo data?), defined category (selection strategy unclear, too small, and only a single negative in vivo), validity of selected NAM (what is their link to the claimed endpoint, what is their metabolic competence, and performance as sensitivity, and specificity?), no worked out AOP (an AOP would have helped substantiating choice assays), validity of applied toxicokinetic models (description too complex, experimental basis – only VPA in vivo data - too small), and conclusion of the read-across (MHA as negative is not fully supported by the data). The poster will depict the provided responses to these comments.

POSTER 6 Case study 3: Integration of qualitative NAM data into the read-across assessment of alkylated phenols and hydroquinones

Laia Tolosa Pardo[1], Barbara van Vugt[2], Johannes Schimming[3], Sylvia E. Escher[4,] and the Case Study 3 team.

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[4]*Fraunhofer ITEM, Germany*

Abstract:

EU-ToxRisk Case Study 3 is designed to explore the toxic behaviour of phenolic compounds (mostly acting via *redox cycling* in cells), and to anticipate the toxicity of related compounds in a *read-across* exercise. In the course of in vitro testing it became noticeable cross-contamination of neighbouring wells, an issue that strongly interfered in the experimental outcomes, and could be related to volatility and gas diffusion of parent compounds or oxidised derivatives. The use of non-permeable seals avoided cross-contamination and allowed us to detect ROS production and effects in viability by *High-content imaging* (*HCI*) in HepG2 cells after both 3 and 24h treatment. Under sealed conditions, also reporter activation was measured in the high throughput assays, Calux and GFP. These data allow to investigate similarity of the compounds relative to each other based on biological data. The analysis of potency differences within the compounds remains questionable, as mass spectrometry analyses revealed evaporation of the compounds under sealed conditions as well as instability in medium.

POSTER 7 Assessment of the toxicity of volatile and/or oxidisable compounds "In vitro: phenols as an example

José Castell[1], Laia Tolosa Pardo[1]

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Abstract:

Phenolic compounds are widely used in industry and are present in many trade products as well as in pollutants in the environment. EU-ToxRisk Case Study 3 was designed to explore the toxic behaviour of these compounds (mostly acting via redox cycling in cells) and to anticipate the toxicity of related compounds in a read-across exercise. Phenols are difficult to handle because of degradation, oxidation and volatility that result in diffusion to the neighbour's wells and the presence of degradation compounds in the assay, which potentially include hydroquinones, quinones and other more complex and polymeric compounds. First experiments run with model phenols trimethylhydroquinone and 2,6-di-tertbutyl-4-ethylphenol to asses cross contaminations disclosed both, the presence of the parent compound in neighbouring wells which was evidenced by Gas Chromatography–Mass Spectrometry (GC-MS) analysis as well by effects on nearby cells. The stoichiometric balance also revealed that the concentration of the compounds in wells decreased, not only by air-diffusion to other wells but also due to oxidation processes. The exhaustive analysis of the phenomenon revealed that Trimethylhydroquinone presented fast degradation kinetics and significant diffusion rates, some of the degradation products being able to air-diffuse as well. We could identify up to 6 degradation products, formed in the course of incubations what indicated that the effects measured on cells are not due only to the parent phenol, thus questioning the in vitro data obtained. On the other hand, 2,6-di-tertbutyl-4-ethylphenol showed lower degradation kinetics, but a high diffusion rate, pointing out that in this case the toxicity could be underestimated because of decrease in concentration of the parent compound, in addition to cross-contamination to other neighbouring wells. To overcome such problems and to make the in vitro study of the toxicity of phenols feasible, we adopted a strategy consisting in the use of gas-permeable and gas non-permeable plastic seals that were placed on the top of multi-well plates replacing the usual plastic lid. Gas non-permeable was expected to prevent diffusion and oxidation of phenols, buy might have drawbacks for the oxygen/CO2 exchange and negatively influence cells. Indeed, diffusion was greatly prevented by the use of plastic seals, as revealed by GC_MS analysis of the content of the neighbouring wells. Gas non-permeable plastic seals, reduced to a minimum diffusion as well oxidation. When experiments were run with cultured wells, cell functionality in control cells was not affected by the plastic seals and no cross-contamination effects were observed in neighbouring wells. Based on these findings we need to emphasize that volatile and/or oxidisable compounds should not be assessed under conventional in vitro incubation conditions, but rather in gas non-permeable plastic film covered wells.

POSTER 8 Case study 4 (CS4): Differential neurotoxicity of a panel of mitochondrial respiratory chain inhibitor pesticides

Anna Forsby[1,2], Andrea Cediel Ulloa[2], Vesna Munic Kos[2], Kristina Attoff[1,2], Johannes Delp[1], Marcel Leist[3]

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[3]*University of Konstanz, Germany*

Abstract:

Specific neuronal subpopulations are particularly sensitive to inhibitors of mitochondrial respiration, as described in detail in the advanced AOP that the EU-ToxRisk project has been working on (https://aopwiki.org/aops/3). Within CS4, we further explored mechanisms relevant to this AOP. To delineate the applicability limits of the AOP, 22 mitochondrial inhibitors have been studied. Different tests have been compared in their ability to assess key events of the above AOP. The tests are based on the human neuronal dopaminergic LUHMES and SH-SY5Y cell lines. Although all tested compounds (complex I and III inhibitors being much more potent) impaired cellular respiration and depolarized the mitochondrial membrane potential (MMP) in LUHMES and SH-SY5Y cells (this reflects MIE, KE1, KE2) at non-cytotoxic concentrations, only a subgroup affected the neurite structures of LUHMES or SH-SY5Y cells (reflects KE4). The clearest neurite-specific effects were observed for rotenone, followed by deguelin. Neurite degeneration in LUHMES and SH-SY5Y cells was accompanied with a decrease in gene-expression of the dopamine-synthesizing enzyme dopa decarboxylase. As stipulated by the AOP, most complex I (cI) inhibitors reduced neuronal viability in LUHMES and SH-SY5Y cells. Most cIII inhibitors were less potent than cI inhibitors in both systems, but still toxic. Only minor effects were observed for cII inhibitors. The data suggest that cell-based neurotoxicity assays may be used to assess KE4 of the AOP, i.e. “degeneration of dopaminergic neurons of nigrostriatal pathway”, and other AOPs may be required for non-cI inhibitors.

POSTER 9 (speed presentation) Identification of mitochondrial toxicants by combined *in silico* and *in vitro* studies – A structure-based view on the Adverse Outcome Pathway

Florentina Troger[1], Johannes Delp[2], Melina Funke[2], Wanda van der Stel[3], Claire Colas[1], Marcel Leist[2], Bob van de Water[3], Gerhard F. Ecker[1]

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[3]*LACDR, Leiden University, The Netherlands*

Abstract:

Drugs that modulate mitochondrial function can cause severe adverse effects. Unfortunately, mitochondrial toxicity is often not detected in animal models, which stresses the need for predictive in silico approaches. In this study we present a model for predicting mitochondrial toxicity focusing on human mitochondrial respiratory complex I (CI) inhibition by combining structure-based methods with machine learning. The structure-based studies are based on CI inhibition by the pesticide rotenone, which is known to induce parkinsonian motor deficits, and its analogue deguelin. After predicting a common binding mode for these two compounds using induced-fit docking, two structure-based pharmacophore models were created and used for virtual screening of DrugBank and the Chemspace library. The hit list was further refined by three different machine learning models, and the top ranked compounds were selected for experimental testing. Using a tiered approach, the compounds were tested in three distinct in vitro assays, which led to the identification of three specific CI inhibitors. These results demonstrate that risk assessment and hazard analysis can benefit from combining structure-based methods and machine learning.

POSTER 10 D6.2: Repository of cell signalling perturbations - A report on the effect of project chemicals on different cell signalling Luc reporter assays

Barbara M. A. van Vugt-Lussenburg[1], H. Y. Man[1], F. Niamut[1], B. van der Burg[1]

[1]*BioDetection Systems bv, The Netherlands*

Abstract:

Within EU-ToxRisk, case studies are used to support the development of AOP-based IATA strategies. Experimental data is generated using non-animal test systems that are able to provide quantitative information on key event (KE) activation. KE activation through modulation of cell signaling responses by chemicals can be evaluated using reporter gene assays; the human cell based CALUX reporter gene assay panel is ideally suited to study the interaction of a compound with a specific receptor or pathway, without interference of metabolism or receptor cross-talk.

The CALUX reporter gene assay panel, consisting of 27 assays, has been used to generate activity profiles of 88 case study chemicals (CS1 – CS5). In deliverable report D6.2, the results of these analyses are summarised, and the suitability of the CALUX panel for the overall aim of the EU ToxRisk case studies is evaluated: to provide proof-of-concept for the applicability of a new approach methodology (NAM) approach in risk assessment.

POSTER 11 Learnings from regulatory feedback on the two read-across case studies on mitochondrial toxicity

Susanne H. Bennekou[1], Andrew White[2], Anna Forsby[3], Barbara Zdradil[4], Bart van der Burg[5], Giada Carta[6], Gerhard Ecker[4], Gladys Ouedraogo[7], Hennicke Kamp[8], Iain Gardner[9], Ciaran Fisher[9], Johannes Delp[10], José Castell[11], José Gomez[12], Manuel Pastor[12], Marcel Leist[10], Paul Jennings[4], Paul Walker[5], Stefan Schildknecht[13], Sylvia Escher[14], Dinant Kroese[15], Martin Moné[13], Wanda van der Stel[13], Bob van de Water[13]

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Abstract:

Both the EU-ToxRisk RAB and the OECD case study project "Integrated Approaches to Testing and Assessment (IATA)" reviewed four RAX case studies from EU-ToxRisk, amongst the two on mitochondrial toxicity. This poster will outline the main findings/comments from the different OECD countries, the OECD and the ICAPO.

Highlights: There was appreciation of the applied AOP-based testing strategy. In this regard, particular learnings could be applied on how the matureness of the AOPs and the number of Key Events that would need testing, especially in a case where the in vivo study is not sensitive to the MoA investigated. The usefulness of also testing reference compounds was appreciated. A biological read-across, i.e. the structural similarity between the target and the source compounds was less, could be supported by this means. PBPK modelling was viewed as strong support for the cases. The case studies prompted a more general discussion on application of NAMs and reporting for regulatory purposes on where to develop more guidance amongst others, which will also be presented. – The case study was accepted for publication by OECD in 2020.

POSTER 12 (speed presentation) Transcriptional responses to ETC inhibition in cultured human renal epithelial cells

Giada Carta[1], Harper VanSteenhouse[2], Paul Jennings[1]

[1]*Vrije Universiteit Amsterdam, The Netherlands*
[2]*Bioclavis, United Kingdom*

Abstract:

The analysis of changes in gene expression upon chemical exposure, provides in depth mechanistic information on the compound's mode of action, by revealing specific pathway activation and modulation. Here we aim to define a transcriptional signature of mitochondrial toxicity induced by inhibition of electron transport chain (ETC). To this end we performed the TempO-Seq assay in human proximal tubule epithelial cells (RPTEC/TERT1) exposed for 24 h to a range of concentrations of a set of complex I, complex II and complex III ETC inhibitors (CI, CII and CIII respectively) for which an energy metabolic profile and cytotoxic range has been already established using the same exposure regime. Differential genes expression (DEG) analysis reveals different patterns for different type of inhibitions. CI reveals a concentration dependent increase in the number of DEG at non cytotoxic concentration, no chances in DEGs were associated with CII whereas CIII had an impact on the high-cytotoxic concentrations. A substantial overlap was found when comparing DEGs for CI vs CIII. Pathway analysis indicates a major involvement of the unfolded protein response pathway (UPR), specifically the ATF4 pathway, in response to the ETC inhibition. Surprising the oxidative stress response pathway, Nrf2, was not activated in contrast with the common reporting of ROS increase upon ETC inhibition. The results indicate the ATF4 pathway as the major adaptive response to ETC inhibition in renal epithelial cells.

POSTER 13 Multiparametric assessment of mitochondrial electron transport chain inhibition in HepG2 and RPTEC/TERT1 cells using a panel of 20 agrochemicals

Giada Carta[1], Wanda van der Stel[2], Julie Eakins[3], Johannes Delp[4], Anna Forsby[5], Susanne Hougaard Bennekou[6], Iain Gardner[7], Marcel Leist[4], Erik H.J. Danen[1], Paul Walker[3], Bob van de Water[2], Paul Jennings[1]

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Abstract:

Mitochondrial perturbations play a key role in the aetiology of several diseases, including metabolic diseases, accelerated aging, certain neurodegenerative diseases and in certain xenobiotic induced organ toxicity. Understanding the relationship between mode of action and tissue sensitivity is fundamental for improving methodologies for the prediction of toxicity and safety assessment. Assessing mitochondrial respiration is not trivial and the outcomes of such investigations is very much dependent on the cell types used and assays employed. Here we systematically investigated the effect of Electron Transport Chain (ETC) inhibitors on multiple mitochondrial related parameters in two human cell types, HepG2 and RPTEC/TERT1, exposed a wide range of concentrations of 21 proposed ETC complex I (CI), complex II (CII) and complex III (CIII) inhibitors. A battery of tests was utilised including viability assays, lactate production, mitochondrial membrane potential (MMP), extracellular acidification rate [ECAR] and oxygen consumption rate [OCR]. CI inhibition caused a dramatic decrease in basal and maximal OCR, increased ECAR, induced 24h lactate production and decreased mitochondrial membrane potential in both cell types. Galactose containing medium sensitized HepG2 cells towards mitochondrial toxicity but not RPTEC/TERT1. CII inhibition had no notable effects in intact cells. CIII inhibitor and had similar effects to the CI inhibitors. The study provides an example of a mitochondrial assessment workflow and establishes key events of ECT inhibition.

POSTER 14 Unravelling the complex response to mitochondrial perturbation and identification of candidate selective biomarker using TempO-Seq technology

Wanda van der Stel[1], Huan Yang[1], Salihanur Darici[1], Harper VanSteenhouse[2], Sylvia Dévédec[1], Bob van de Water[1], Joost Beltman[1], Erik H.J Danen[1]

[1]*LACDR, Leiden University, The Netherlands*
[2]*Bioclavis, United Kingdom*

Abstract:

Mitochondrial perturbation is a key player in chemical-induced organ toxicities. Mitochondria-associated toxicity is therefore part of the general assessment pipeline for chemical legislation and drug development. However, this assessment does not incorporate any evaluate of possible differences between mitochondrial toxicant classes or the mode of action. This particular information could feed the toxicity prediction of new chemicals and drugs and would therefore be a valuable addition to the standard mitochondrial perturbation pipeline. Here we studied the effects on Electron Transport Chain (ETC) inhibitors on mitochondrial perturbation and the associated toxicity mechanisms using a high throughput screening platform assessing both biochemical read outs using high content imaging, and cellular-mitochondrial signalling using the TempO-Seq technology. Complex I and III inhibitors perturbed mitochondrial integrity and decreased cellular viability in a concentration and time dependent fashion whereas complex II inhibitors had no effect. Overall changes in gene expression corresponded to MMP perturbation and GO-term and transcription factor enrichment studies revealed a major effect upon cell proliferation, but also the upregulation of cellular stress responses and especially ATF4-related target genes. Analyses of effects on MMP, viability, adaptive stress related proteins, and GO analysis did not identify markers distinguishing complex I and III ETC inhibitors. However, a panel of 8 genes was identified that was specifically induced by active ETC inhibitors and not in response to genotoxic, oxidative, or ER stress. Next steps will include validation of this gene set in primary liver cells, and an assessment of their involvement in direct/indirect perturbation of mitochondria using KD studies. Altogether, we unravel quantitative biochemical and signalling responses to mitochondrial perturbation and identify a selective gene. This set of genes can represent a candidate biomarker for early stages of mitochondria-related toxicity.

POSTER 15 An intra model comparison of rotenone exposure using the TempO-Seq platform

Wanda van der Stel[1], Giada Carta[2], Johannes Delp[3], Anna Forsby[4], Harper VanSteenhouse[5], Erik Danen[1], Marcel Leist[3], Paul Jennings[2], Bob van de Water[1]

[1]LACDR, Leiden University, The Netherlands; [2]Vrije Universiteit Amsterdam, The Netherlands; [3]University of Konstanz, Germany; [4]Stockholm University, Sweden; [5]Bioclavis, United Kingdom.

Abstract:

A variety of epidemiological studies demonstrate a correlation between pesticide exposure and development of Parkinson liabilities. This occurrence of Parkinson liabilities has only been observed upon exposure to chemicals which perturb the mitochondrial electron chain complex I (ETC CI), among others Rotenone. To be able to improve understanding of the causal relationship between mitochondrial perturbation and neuron degradation and to assess the possible tissue specific responses, we employed a transcriptomic assessment using TempO-seq technology in 4 cell lines – HepG2, RPTEC/TERT1, LUHMES and SH-SY5Y –.

A analysis of ~3000 genes – involving components of adaptive stress pathways and toxicity responses, but also cell type specific markers – identified up and down regulation of gene sets independently of the used cell line, but also hits unique for neuronal tissue type. A further comparative analysis including concentration response data for all cell lines will be discussed.

All together monitoring cell- and chemical-specific transcriptomic fingerprints could improve understanding of cell lines specific responses to ETC inhibition and provide quantitative information to support risk assessment in the future.

POSTER 16 Case Study 5 – phase 1 testing of peroxisome proliferators for in vitro read across

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Abstract:

The phenoxy acetic/ propionic acid herbicides form a group of structurally similar herbicides that have been shown to induce similar systemic toxicity in rat studies. Main toxicological effects observed are liver toxicity due to peroxisome proliferation as well as kidney toxicity associated with oxidative stress. Within case study 5, different test systems and read-outs (e.g. CALUX reporter gene assays, HepG2 metabolomics and stress response, RPTEC/TERT1 stress response as well as transcriptomics in the different cell systems) will be used to show biological similarity in vitro, which can be used for a NAM-based read across. The first experimental phases have been finalized and data from the CALUX assays, hepG2 metabolomics and stress response show that the biological effects observed can be linked to the toxicological mode of action in the liver. In a principal component analyses, a clear separation for the herbicides as well as MEHP from the control treatments along the first principal component was observed based on the in vitro metabolome data. The metabolite profile indicates changes in lipid metabolism as can be seen for peroxisome proliferators in vivo. The data for the herbicides are well in line with the in vivo data as published in van Ravenzwaay et al., 2016) and show that these in vitro data might be used to substantiate a read across based on in vitro methodologies.

POSTER 17 Effect of peroxisome proliferator-activated receptor (PPAR) activators on mitochondrial respiration rates in cultured human hepatocyte cells (HepaRG)

Tamara Meijer[1], Liliana Capinha[1], Anja Wilmes[1], and Paul Jennings[1]

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Abstract:

Mitochondria are of crucial importance in the production of adenosine triphosphate (ATP) through oxidative phosphorylation. The aim of this study was to investigate the potential effects of the 21 Case-Study-5 compounds on mitochondrial respiration and glycolysis in cultured HepaRG cells. Cells were treated for 24 hours with concentrations up to 100 µM. The Agilent Seahorse XF Technology platform was used to quantify oxygen consumption rates and extracellular acidification rates. The results revealed that simvastatin, atorvastatin, clofibrate, bezafibrate and pioglitazone all exhibit an effect on mitochondrial respiration at 100 µM or below. Bezafibrate and clofibrate increased basal respiration rates, which may be related to PPAR activation. The data and implications are presented.

POSTER 18 CS8: The popcorn lung – overview and experimental challenges

Tanja Hansen[1], Jan Boei[2], Harry Vrieling[2], Johannes Schimming[2], Stefanie Klima[1], Sylvia Escher[1], Katherina Sewald[1], Jan Knebel[1], Detlef Ritter[1]

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[2]*Leiden University Medical Center (LUMC), The Netherlands*

Abstract:

Alpha-diketones such as diacetyl (2,3-butadiene) are known to induce the so called “popcorn lung”, an obstructive pulmonary disease in which the airway epithelium is the initial target of injury. Besides four -diketones, one β- and one γ-diketone as well as two volatile reference compounds without known adverse respiratory effects are included into this case study. The volatility of the case study compounds leads to difficulties in establishing a quantitative dose-response relationship when in vitro models are exposed under submerged conditions. Here we present the results of submerse neurotoxicity testing and HepG2 reporter assays. Finally, we show an optimized in vitro exposure setup for the exposure of cell cultures and precision cut lung slices (PCLuS) to volatile compounds at the air-liquid interface.

POSTER 19 Hazard assessment of diacetyl and structurally related diketones – a read-across approach

Tanja Hansen[1], Matthias Wehr[1], Britta Anna Kühne[1], Detlef Ritter[1], Jan Knebel[1], Johannes Schimming[2], Jan Boei[2], Pieter S. Hiemstra[2], Harry Vrieling[2], Sylvia E. Escher[1]

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Primary human bronchial epithelial cells (PBECs) were isolated from tumor-free lung tissues from four donors and differentiated into mucociliary epithelial cells at air-liquid interface (ALI) conditions. The cells were exposed to the case study chemicals under (ALI) conditions using the P.R.I.T.® ExpoCube® device for 1h once or repeatedly on three consecutive days. Cellular viability was measured by LDH-leakage and barrier function by measuring the transepithelial electrical resistance (TEER) 24h after the final exposure. Exposure concentrations ranged from 100 to 1840 ppm (diacetyl) and from 50 to 5000 ppm (other diketone analogues). Chemical-induced transcriptomic responses were investigated utilizing targeted RNAseq with the Templated Oligo Detection Assay (TempO-Seq) based on a 3347 gene panel. TempO-Seq analysis revealed up or down regulated differentially expressed genes (DEGs) in a dose and exposure time dependent manner. Analysis of the gene expression patterns indicated that some of these diketones may share a similar mode of action. Translational analyses were carried out to link these in vitro data to relevant adverse human outcomes like pulmonary fibrosis and inflammation.

POSTER 20 New insights in Read Across using New Approach Methods: illustration with the propyl paraben case study

C. Mahony[1], S. Stuard[1], J. Naciff[1], C. Ellison[1], B. Desprez[2], M. Klaric[2], A. Detroyer[3], N. Hewitt[2], A. Schepky[4], Christopher-Tilman Krüger[4], D. Bury[3], Evita Vandenbossche[5], M. Dent[5], G. Kenna[2], D. Keller[6], M. Cronin[7], A. Bitsch[8], E. Mombelli[9], T. Cull[5], B. van der Burg[10], G. Ouédraogo[3]*

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Abstract:

In Read Across, untargeted and targeted NAMs can help to strengthen the identification of suitable substances for read across, increasing confidence in the NOEL used as a POD for the risk assessment. NAMS have also been used to inform on relative potency of analogue mode of action and to predict internal exposure in both human and animal studies allowing for a risk assessment approach based on internal exposures of the human versus the animal study. The considerations in following this 'next generation Read Across' approach are presented here, and illustrated by the joint Cosmetics Europe-EuToxRisk parabens case study.

POSTER 21 Next generation risk assessment of developmental neurotoxicity liabilities of neonicotinoids insecticides

Susanne H. Bennekou[1], Anna Forsby[2], Ylva Johansson[2], Maria G.H. Hidalgo[2], Barbara Zdradil[3], Bart van der Burg[4], Barbara van Vugt-Lussenburg[4], Iain Gardner[5], Andras Dinnyes[6], Zofia Ajnstova[6], Jose C. Gomez[7], Manuel Pastor[7], Paul Walker[5], Jonathan Blum[8], Oana Florean[9], Rebecca von Hellfeld[10], Thomas Braunbeck[10], Marcel Leist[8]

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Abstract:

The different regulatory sectors historical use of the in vivo DNT test guideline (OECD TG426) has been limited, the challenges being a lack of DNT data for most chemicals, lack of mechanistic information, and difficulties in interpreting the animal results for their relevance to humans. This case study will be one of the case studies supporting the future "OECD DNT guidance on interpretation of in-vitro DNT data that can be used in an IATA", which is currently being developed.

This case study aims to develop an IATA on the neonicotinoid pesticides, imidacloprid and acetamiprid, that could support hazard identification/characterization. This will be done by testing of a number of neonicotinoid compounds. The objectives and questions defining the case study, the approach chosen, the different assays and models will be presented.

POSTER 22 Assembling knowledge in a DNT AOP to provide biological context for Neonicotinoids

Oana C. Florean[1], Barbara Zdrazil[2]

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[2]*University of Vienna, Austria*

Abstract:

Adverse Outcome Pathway (AOP) is a knowledge organizing concept having an innovative potential in the risk assessment field. AOPs describe toxicological modes and mechanisms of action in an effective way. They are represented as schemes, built from the most important events found in different levels of biological organization. The sequence of AOP components starts with a molecular initiating event (MIE), continues with a number of key events (KE) and ends with an adverse effect (AO).

In the present study, we attempt to assemble an AOP which links the activation of nicotinic acetylcholine receptors (nAChRs) by nicotine and neonicotinoids compounds to developmental neurotoxicity (DNT) effects. We attempted to build the AOP by using any type of data that is available on the compounds and empirically bringing everything together. Because few and heterogenous omics data was found on the case study compounds, evidence about the compounds regarding their DNT effects was collected from relevant literature. Events from already published AOPs were also taken in consideration for integration into the AOP. Furthermore, the actual configuration of the AOP will be complemented with insights from the case study experimental results where possible.

As a second line of evidence we are performing searches in public data sources (e.g. the ChEMBL database). Target profiles of Case Study compounds and structurally similar compounds are analyzed and the information is joined with the manual searches in order to prioritize potential key events at the level of protein targets.

POSTER 23 (speed presentation) Effect of a subgroup of neonicotinoid insecticides on human neuronal function

Jonathan Blum[1], Dominik Loser[2], Barbara van Vugt-Lussenburg[3], Bart van der Burg[3], Maria Hidalgo[4], Ylva Johansson[4], Anna Forsby[4], Andras Dinnyes[5], Susanne Hougaard Bennekou[6], Marcel Leist[1]

[1]University of Konstanz, Germany; [2]Naturwissenschaftliches und Medizinisches Institut, Germany; [3]BioDetection systems bv, The Netherlands; [4]SweTox Unit for Toxicological Sciences, Stockholm, Sweden; [5]BioTalentum Ltd., Hungary; [6]National Food Institute, Technial University of Denmark, Denmark.

Abstract:

Developmental neurotoxicity (DNT) is one of the least tested effects of chemicals. This lack of testing is mainly attributed to the issue, that current DNT assessment is entirely based on in vivo animal experiments. Therefore, new approach methods (NAMs) have been recommended in the past few years to contribute to the test status of DNT. In 2013 the environmental food safety agency (EFSA) released a scientific opinion raising concerns regarding the DNT potential of two neonicotinoid pesticides, acetamiprid and imidacloprid. Literature research revealed that the whole subgroup of neonicotinoid insecticides lack DNT data and mechanistic information resulting in difficulties to interpret the available results for risk assessment. Here, we present data of a combined NAM test battery including cell-based assays and model organisms like zebrafish. We found, that most tested endpoints were not affected by the treatment with neonicotinoids. However, Ca2+ influx measurements as an example revealed alterations comparable to the well-known DNT compound nicotine. Taken together the results across the battery expand the knowledge of available in vivo data and could therefore contribute to a better risk assessment.

POSTER 24 (speed presentation) Kinetic analysis of trichloroethylene glutathione conjugates and the effect of their metabolites on mitochondrial respiration using cultured human renal proximal tubular cells

Liliana Capinha[1], Jan N.M. Commandeur[1], and Paul Jennings[1]

[1]*Vrije Universiteit Amsterdam, The Netherlands*

Abstract:

Trichloroethylene (TCE) is a high-production volume chemical that has been widely used for decades and poses a significant hazard to human health. Animal and in vitro studies have demonstrated that hepatic glutathione conjugation of TCE by glutathione S-transferases (GST) and subsequent renal metabolism involving ϒ-glutamyl-transferase (GGT), cysteinylglycinase and cysteine conjugate β-lyase activity can lead to the formation of a reactive thioketene, causing oxidative stress and mitochondrial injury.

Here, we re-examined the hepatic GSH-conjugation of TCE and its effects on human renal proximal tubule cells. By using human liver fractions and recombinant human GSTs we demonstrate the formation of trans 1,2-dichlorovinyl-glutathione (trans 1,2 DCVG) and 2,2-dichlorovinyl-glutathione (2,2 DCVG). RPTEC/TERT1, were differentiated and treated with trans 1,2 DCVG and 2,2 DCVG. A fast and complete metabolism of the initial GSH-conjugates was observed within 2 hours, with a temporal transient increase of the respective cysteine-glycine conjugate (DCV-cys-gly), followed by the formation of the cysteine conjugate (DCVC). LC-MS-TOF was used to monitor the biotransformation of the GSH-conjugates over 24 h. The specific effects of the two GSH-regioisomer conjugates on mitochondrial respiration were quantified by using a Seahorse bioanalyser. A decreased in oxygen consumption rate (OCR) and redox capacity was observed for trans 1,2 DCVG, but not in the presence of β-lyase inhibitor aminooxyacetic acid (AOAA). 2,2 DCVG had no effect.

This study provides new insight regarding the molecular processes of TCE metabolism and toxicity in human renal cells demonstrating a clear relationship between metabolism and isomer-conjugate dependent mitochondrial perturbation.

POSTER 25 Cosmetics Europe's Long Range Science Strategy: Update on the Non-Animal Safety Assessment Case Study of Phenoxyethanol for cosmetics

E. Vandenbossche[3], M. Nepelska[3], M. Cronin[2], R. Cubberley[3], M. Dent[3], B. Desprez[1], J. Firman[2], J. Fisher[4], G. Kenna[7], C. Mahony[5], B. Nicol[3], S. Piechota[3], K. Przybylak[3], A. Schepky[6], S. Tozer[5], J. Troutman[4], CE. Hack[8], M. Klaric[1*]

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Abstract:

We present an update on the Cosmetics Europe LRSS exposure-based ab initio case study for the cosmetic ingredient phenoxyethanol. The case study was guided by the SEURAT-1 safety assessment workflow (Berggren et al (2017) and the International Cooperation on Cosmetics Regulation NGRA principles (Dent et al., 2018), with the ambitious aim of using only non-animal approaches to assure the systemic safety of phenoxyethanol when present at an active levels (1%) in a product with a high level of consumer use (body lotion). This strategy is aligned with the US EPA's next generation blueprint for toxicology, which seeks to characterize whether a chemical acts via defined biological pathways/targets or if it may induce cellular changes by a non-specific mechanism (Thomas et al., 2019)

POSTER 26 Addressing scientific challenges associated with ab initio chemical safety assessment

Donatella Carpi[1], Thomas Cole[1], Salvador Fortaner Torrent[1], Peter Macko[1], Alicia Paini[1], Taina Palosaari[1], Jukka Sund[1,] and Maurice Whelan[1]

[1]*European Commission – Joint Research Centre*

Abstract:

The EU funded SEURAT-1 project used the term ab initio to refer to data-poor chemical safety assessment using non-animal methods (Berggren et al., 2017). To face the evaluation of chemicals without relying on animal data to directly "validate" against, a tiered workflow was proposed consisting of a stepwise, investigative-type process with hypothesis driven data generation and analysis, supported by characterisation of uncertainty at each step. Following on from this work, the ab initio workshop organised by JRC, EU-ToxRisk and Cosmetics Europe in April 2019 (report available) identified three key scientific challenges to be addressed: biokinetic and metabolism prediction; formulation of mode-of-action hypotheses (e.g. to direct targeted testing); and definition of margin-of-safety (e.g. taking potency, severity and reversibility of effect into account). These challenges are being tackled in the EU-ToxRisk LowTox Case Study where, after accurate definition of the assessment scenario (problem formulation), data-poor chemicals are investigated individually or in small groups through chemical-specific assessment strategies that include in silico modelling, biokinetic profiling and targeted in vitro bioassay. At each step, an attempt is made to answer the specific safety question, and then the next assessment step is defined based on the uncertainties identified.

POSTER 27 (speed presentation) Unilever's vision on next generation risk assessment (NGRA)

Andrew White[1] et al.

[1]*Unilever, United Kingdom*

Abstract:

Not available

POSTER 28 BioStudies database for project data management and sharing

Ugis Sarkans[1], Nestor Diaz[1], Anne Hersey[1], Francis Atkinson[1], Thomas Exner[2], Daniel Bachler[2]

[1]*European Bioinformatics Institute (EMBL-EBI), United Kingdom*
[2]*Edelweiss Connect GmbH, Switzerland*

Abstract:

BioStudies is a database at EMBL-EBI that facilitates transparency and reproducibility of research, by aggregating all the outputs of a study (a 'data package') in a single place. BioStudies is used in EU-ToxRisk as a primary data sharing platform. Project partners upload their *in vitro* and *in silico* data into BioStudies, according to well-defined data reporting templates. Datasets can be accessed directly through BioStudies, and ToxDataExplorer (and potentially other tools in the EU-ToxRisk Knowledge Infrastructure) can import data for further processing and visualization. More than 1000 datasets are available as of January 2020.

POSTER 29 EU-ToxRisk knowledge infrastructure 2020 - effective sharing of data, results and knowledge

Thomas Exner[1], Maja Brajnik[1], Lucian Farcal[1], Ugis Sarkans[2], Anne Hersey[2]

[1]*Douglas Connect GmbH, Switzerland*

[2]*European Bioinformatics Institute (EMBL-EBI), United Kingdom*

Abstract:

For the effective sharing of data, results and knowledge, EU-ToxRisk has established a knowledge infrastructure continuously developed and improved to optimally support the work in the work packages and especially the case studies. It builds the link between the data and tool providers, on one hand, and risk assessors, on the other hand, as the consumers of the data and users of the provided tools.

EU-ToxRisk KI consists of different modules that include:

- Long-term data storage solutions;
- Linked visualisation and modelling tools;
- Test method and in silico methods repositories;
- Case studies and AOP collaborative sections.

In this year's poster, the improvements in the data management based on a new version of the ToxDataExplorer, better data access options using a python library, the updates on the test methods also regarding semantic annotation and the case study management module will be presented. All this can also be looked at in a live demo.

POSTER 30 (speed presentation) QSAR modelling of in vitro intrinsic clearance measured in human hepatocytes

Enrico Mombelli[1], Domenico Gadaleta[2], Cosimo Toma[2], Alessandra Roncaglioni[2], Iain Gardner[3], Ciarán Fisher[3], Emilio Benfenati[2]

[1]*Institut National de l'Environnement Industriel et des Risques (INERIS), France*
[2]*Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Italy*
[3]*Certara UK Limited, Simcyp Division, United Kingdom*

Abstract:

Intrinsic clearance (CLint; µl/min/106 cells) measured in human hepatocytes is used to gain an insight into the toxicokinetics properties of xenobiotics and is a vital parameter for PBPK modelling and simulation. This property describes the capability of the liver to remove chemicals in the absence of flow limitations and binding to cells or proteins in the blood. Prediction of intrinsic clearance using in silico tools would represent a practical advantage in the application of PBPK in toxicology chemical risk assessment. For this reason, the purpose of our work was to implement Quantitative Structure-Activity Relationships (QSAR) models aimed at predicting CLint.

QSAR models were derived as a function of three algorithms: Random Forests, XGBOOST, and Support Vector Machines (SVM). Individual QSAR models were also combined within integrated models to make an intelligent use of multiple QSAR. Before modeling data collected on CLint were corrected for unspecific binding to hepatocytes.

All the models are robust and that there is a gain in performance when the correction for unspecific binding is applied. The CLint of most chemicals can be predicted within a 3-fold and 5-fold error. Nevertheless, our work highlights the difficulty of identifying highly predictive relationships. However, the proposed models, and especially the integrated model, could still be useful for screening level tasks.

POSTER 31 An integrated similarity-based workflow for automated chemical read-across

Domenico Gadaleta[1], Azadi Golbamaki Bakhtyari[1], Giovanna J. Lavado[1], Alessandra Roncaglioni[1], and Emilio Benfenati[1]

[1]*Laboratory of Environmental Chemistry and Toxicology, Department of Environmental Health Sciences, IFRM, Italy*

Abstract:

Read-Across (RAX) is a popular data-gap filling technique that uses category and analogue approaches to predict toxicological endpoints for a target chemical using experimental values of similar compounds (i.e., analog(s)). Despite its increasing relevance for regulatory purposes, currently RAX often relies on human expert judgement and lacks a subjective and automated protocol for the identification of suitable source analog(s) for RAX. Here we propose a fully automated procedure for the selection of analog(s) for data gap-filling. Analog(s) were identified by means of a decision algorithm that integrates three similarity metrics that consider different toxicologically relevant aspects (i.e. structural, biological and metabolic similarity). Each similarity metric was determined by mean of a tailored algorithm and implemented into a KNIME workflow that was used to automatically compile independent lists of candidate analogues. Compound(s) included in multiple similarity lists are suggested as most suitable analog(s), and their activity is used to infer the activity of the target chemical. Structural filters based on the presence of maximum common substructures (MCS) and common functional groups were also applied to narrow the chemical space for the analog(s) search. The validation made on a series of datasets relative to high-tier in vivo toxicological endpoints confirmed the advantages of integrating multiple similarities with respect of the sole use of chemical similarity, and the benefit of the tool here presented to support regulatory decision-making.

POSTER 32 PBK modelling to support the neo-nicotinoid case study

Iain Gardner[1] and Ciarán Fisher[1]

[1]*Certara UK Limited, Simcyp Division, United Kingdom*

Abstract:

The neo-nicotinoid compounds represent one of the largest classes of insecticides used worldwide. Available physicochemical, biochemical and pharmacokinetic data was collated for Acetamiprid, Clothianidin, Dinotefuran, Imidacloprid, Thiacloprid, Thiamethoxam and the comparator compound Nicotine. The information was used to construct physiologically based kinetic (PBK) models for the different compounds and the results from the models were compared with observed pharmacokinetic data. Where appropriate the PBK models incorporated metabolites of the parent compound (eg for clothianidin formation from thiamethoxam and cotinine formation from nicotine).

The compounds had moderate lipophilicity (log P in the range -0.6 to 1.3), moderate to high solubility and were cleared mainly by metabolism or renal elimination. Protein binding in humans was predicted using an *in silico* model and for compounds where measured values were reported there was good agreement with the predicted values (r2 = 0.91). The predicted brain:plasma ratio was similar in humans and rodents with values in human ranging from 0.58 (dinotefuran) to 2.77 (nicotine). The predicted values were within 2.7-fold of the observed data in rodents for the four compounds where measured data was available (Imidacloprid, Clothianidin, Dinotefuran and Nicotine). The developed PBK models will be used to simulate different exposure scenarios in support of the Neo-nicotinoid case study.

POSTER 33 VIVD: a Virtual In Vitro Distribution model for predicting intra- and sub-cellular concentrations in toxicity assays

Ciarán Fisher[1], Ségolène Siméon[2], Masoud Jamei[1], Frédéric Bois[1], Iain Gardner[1]

[1]*Certara UK Limited, Simcyp Division, United Kingdom*
[2]*Institut National de l'Environnement Industriel et des Risques (INERIS), France*

Abstract:

In vitro testing routinely uses nominal treatment concentrations as the driver for measured toxicity endpoints. However, test compounds can bind to the plastic of culture vessels or interact with culture media components, such as lipids and albumin. Additionally, compounds can partition into the air above culture media. These processes reduce free concentrations of compound to which cells are exposed.

Models predicting the impact of these interactions, and so freely dissolved concentrations, have been published. However, these have only been applied to neutral compounds or assume no significant ionisation of test compounds. Herein, we describe an in vitro distribution model, based on the Fick-Nernst Planck equation, that accounts for differential compound ionisation in culture media and intracellular water, describes permeability of both ionised and unionised species, and accounts for membrane potential in the partitioning of ionised moieties.

The VIVD model provides a steady-state, mechanistic framework for predicting freely dissolved cellular and subcellular concentrations and has been used to generate predictions for > 100 case study compounds as part of the H2020 EUToxRisk project (681002)

POSTER 34 An Imaging-based RNA-interference Screen Reveals Novel Key Regulators of the Drug-induced Endoplasmic Reticulum Stress Response

Marije Niemeijer[1], P. Kartal[1], Giulia Callegaro[1], and Bob van de Water[1]

[1]*LACDR, Leiden University, The Netherlands*

Abstract:

Mechanistic understanding of drug-induced liver injury (DILI) is currently still lacking and therefore hard to predict. Some of these drugs induce endoplasmic reticulum (ER) stress and activate the unfolded protein response. However, during chronic ER stress, activation of the UPR will be insufficient and will activate apoptotic pathways mediated by CHOP leading to hepatotoxicity. To improve mechanistic understanding, we aimed to identify novel key regulators of CHOP. We applied an imaging-based RNAi screen of the druggable genome targeting 3457 genes in HepG2 CHOP-GFP cells to identify novel regulators of the tunicamycin-induced ER stress response. CHOP-GFP expression was evaluated after 16 hours of tunicamycin exposure which was altered by 201 genes upon knockdown. These potential regulators were further evaluated with other ER stress inducers or DILI compounds and their role in induction of other UPR-related genes such as ATF4, XBP1 and BIP. To evaluate the relevance of 10 selected novel regulators for the human liver during ER stress, we evaluated the transcriptome in both HepG2 and primary human hepatocytes (PHHs) after knockdown and subsequent exposure for 16 hours of tunicamycin. Three potential regulators were confirmed in PHHs which showed upon knockdown perturbation of UPR activation after tunicamycin. Pathway analysis revealed their key role in multiple ER signalling pathways, namely translation, protein degradation, UPR and protein trafficking. Overall, our RNAi screen allowed the identification of novel regulators of the drug-induced ER stress response and will further shape our understanding and prediction of DILI liabilities.

POSTER 35 (speed presentation) Consolidation of Gene Network Regulation in Different Liver Test Systems by Drugs with Hepatotoxic Liabilities

Lukas Wijaya[1], Giulia Callegaro[1], Steven Kunnen[1], Marcel Leist[2], Jan Hengstler[3], Hennicke Kamp[4], James Stevens[1], Sylvia le Devédéc[1], Bob van de Water[1]

[1]LACDR, Leiden University, The Netherlands
[2]University of Konstanz, Germany
[3]Leibniz Research Centre for Working Environment and Human Factors, Germany
[4]BASF SE, Germany

Abstract:

Drug-induced liver injury (DILI) is the most prevalent adversity encountered in drug development and in clinical settings. Various approaches have been utilized to predict the occurrence of DILI. However, the mechanistic understanding of DILI remains unclear due to the complexity of cellular responses during its occurrence. Previous studies have reported that the cellular responses upon toxic compound exposures are modulated at the gene levels. Thereby, transcriptomic profiling of the cellular responses in hepatocyte model cell lines upon hepatotoxic compound exposures potentially unravel exhibit the prominent cellular responses during DILI episode. In this study, 22 drugs including 14 DILI compounds and 8 non-DILI compounds causing cellular perturbations were investigated. The exposure was performed in human liver carcinoma (HepG2) cell line with 6 different concentration levels. The cells were lysed at 8 and 24 hours after exposure and the lysate was processed with high throughput transcriptomic (TempO-Seq-BioSpyder) pipeline covering the whole genome library. The read count was normalized based on the count per million and the log2 fold change value was calculated utilizing Deseq2 package in R. The obtained gene expression data was analysed for gene network (module) preservation against gene co-expressed modules obtained with primary human hepatocytes (PHH) and for building a de-novo network representing the activated cellular responses with weighted gene correlation network analysis (WGCNA) approach. The results clearly show a meaningful difference in gene expression level between 8 hour and 24-hour exposure with an expected higher number of differential expression genes (DEGs) from 24 hours exposure than from 8 hour exposure. The number of DEGs generally increases upon the increase of DILI compound concentrations, as opposed to non-DILI compounds. Upon the network preservation analysis, the response of HepG2 cells exhibits moderate similarity to PHH especially in the stress responses such as heat shock responses, oxidative stress responses, unfolded protein responses, and DNA damage responses. Furthermore, de novo HepG2 modules representing stress response pathways show high score upon the exposure of DILI compounds with a meaningful dose-response relationship at 24 hours. Additional compounds are now included to establish more refined co-regulated networks that eventually allow to accurately profile the cellular responses upon DILI episodes.

POSTER 36 (speed presentation) Benchmark dose analysis of the transcriptional responses induced by four prototypical stressors in model systems of four different human tissues

Jan Boei[1], Marije Niemeijer[2], Paul Jennings[3], Marcel Leist[4], Bob van de Water[2], and Harry Vrieling[1]

[1]*Leiden University Medical Centre (LUMC), The Netherlands*
[2]*LACDR, Leiden University, The Netherlands*
[3]*Vrije Universiteit Amsterdam, The Netherlands*
[4]*University of Konstanz, Germany*

Abstract:

In order to define the diversity landscape of stress pathway activation among human tissues, four model systems representing lung (PBEC-ALI), kidney (RPTEC), liver (HepG2) and brain (LUHMES) were exposed to a wide dose-range of four prototypical stressors. Twenty-four hours following exposure to CDDO-Me (Nrf2 pathway activation, oxidative stress), Tunicamycin (unfolded protein response, ER stress), Cisplatin (p53 pathway activation, DNA damage response) and TNF (NFkB activation, stress response and inflammation), RNA was isolated and subjected to TempOseq-based transcriptomics using the S1500+ gene set. Subsequent data analysis focused on identification of the "Point of Departure" of individual genes and stress pathway activation through benchmark dose analysis with BMDexpress. In general, transcriptional responses for 'known' stressor-specific genes and related pathways were observed although the benchmark doses for their (in)activation were sometimes remarkably different. Also, the number of responsive genes of a particular pathway varied considerably between the human tissue models. Beside the 'known' genes, many other genes were responsive at low stressor concentrations from which a substantial proportion were unique for a particular organ system, highlighting the diversity of the model systems in their transcriptional responses.

POSTER 37 (speed presentation) Imaging-based single cell dynamics of KEAP1/Nrf2 pathway activation status to quantify pro-oxidant-induced cell fate decisions

Johannes P. Schimming[1], Bas ter Braak[1], Bob van de Water[1]

[1]*LACDR, Leiden University, The Netherlands*

Abstract:

A common mode of action (MoA) involved in drug induced liver injury (DILI) are the formation of reactive oxygen species and radical metabolites. This is exemplified by drugs causing intrinsic DILI like acetaminophen but is also suspected to occur with drugs causing idiosyncratic DILI. The Nuclear factor erythroid 2-related factor 2 (Nrf2) pathway is essential for the cellular defence against such cell damage by orchestrating the oxidative stress response (OSR), i.e. by activation of glutathione synthesis, the thioredoxin system and sulfiredoxines. We want to better understand the dynamics and amplitude of the Nrf2 pathway activation in drug induced liver injury as it may give insights for the liver toxicity liability of chemicals. Here we present a novel approach that allows the assessment of the interplay between the activation of the Nrf2 pathway and cell fate decisions (adaptive vs adverse response). Thus, we created a dual-color HepG2 fluorescence reporter cell line (Nrf2-GFP and SRXN1-mScarlet) to follow the translocation of the transcription factor Nrf2 to the nucleus and the subsequent activation of the transcriptional response by sulfiredoxin 1 (SRXN1) on a single cell level. Through single cell tracking we followed the cellular response upon the exposure to a set of compounds inducing oxidative stress and radical formation. Furthermore, through the preexposure to the Nrf2 activator Bardoxolone methyl (CDDO-me) and the L-buthionine sulfoximine (BSO), an effective inhibitor of glutathione synthetase, we simulated pre-activated state or inhibited state of the OSR. A siRNA knock allowed the biological simulation of the inhibition and activation of the OSR. The dynamical patterns found in the cells under the various condition allowed to judge when an exposure to a compound becomes critical for a cell and subsequently lead to an adverse outcome if correctly extrapolated to the in vivo situation. This detailed characterization of compounds might add great value towards the correct prediction of DILI potential in vitro and if integrated with other MoA driven test systems to an alternative to preclinical safety testing in vivo.

POSTER 38 (speed presentation) Differential HMOX1 oxidative stress reporter activation in different iPSC-derived tissue target cells

Bas ter Braak[1], Kirsten Snijders[1], Anita Fehér[2], Z. Táncos[2], I. Bock[2], Marije Niemeijer[1], Linda van den Berk[1], Julianna Kobolák[2], Anja Wilmes[4], Paul Jennings[4], Catherine Verfaillie[3], András Dinnyés[2], Bob van de Water[1]

[1]LACDR, Leiden University, The Netherlands; [2]BioTalentum Ltd., Hungary; [3]Katholieke Universiteit Leuven, Belgium; [4]Vrije Universiteit Amsterdam, The Netherlands

Abstract:

Cellular stress responses are critical for the repair of injured cells during diverse types of tissue damage, under pathological conditions as well as adverse responses to drug exposures. Targeting cellular stress response activation can contribute to cellular fitness and resilience to stress conditions. We generated an eGFP tagged HMOX1 expressing human induced pluripotent stem cell (hiPSC) line, to visualize the dynamics of oxidative stress on a single cell level. Both endogenous and scar free tagging was achieved using CRISPR/Cas9 technology, resulting in the maintenance of a representative cellular stress response in vitro. HiPSCs are an excellent model for in vitro safety screening since they rapidly divide, have undisrupted metabolic activity and can be differentiated into a whole range of cell types. This overcomes many of the limitations found in current in vitro models such as availability, inter-donor variability and stability. In order to dissect lineage specific oxidative stress response dynamics upon chemical exposure we differentiated the HMOX1-GFP hiPSC reporter into different lineages including hepatocytes, cardiomyocytes, neuronal cells and kidney cells. The various differentiated cells were exposed to different oxidative stressors, CDDO-Me, diethyl maleate and paraquat followed by live cell imaging of the reporter activation using automated high content confocal imaging (HCI). We found large cell type dependent differences in amplitude of the oxidative stress response as well as the dynamic profile as a whole. This provides insights into organ specific sensitivity and dynamics towards chemically-induced oxidative stress response signalling. We anticipate that our hiPSC cellular stress response reporters in combination with HCI may play a key role for refined understanding of the dynamic stress response signalling in diverse target tissue under adverse drug reactions.

POSTER 39 (speed presentation) Selection of novel biomarkers based on co-regulated gene networks to assess adaptive stress pathway activation

Linda van den Berk[1], K.E. Snijders[1], B. ter Braak[1], M. Niemeijer[1], G. Callegaro[1], S.J. Kunnen[1], B. van de Water[1]

[1]*LACDR, Leiden University, The Netherlands*

Abstract:

Development of new drugs, agrochemicals and cosmetic ingredient candidates is still impaired by liabilities for adverse drug reactions, which is accompanied by the activation of diverse cellular stress response activation. Targeting cellular stress response activation, such as ER stress, oxidative stress, DNA damage and NF-κB-mediated inflammation with reporter cell lines can contribute to the early detection of cell adversities upon drug exposure. Given the diversity of mode-of-action of chemicals as well as differences in gene activation in different cell types, there is a need to identify novel specific biomarkers for stress response activation that would cover the diversity of responses. Weighted gene co-network analysis (WGCNA) approaches enable organization of toxicogenomics data and contribute to the discovery of these novel regulators. We used the WGCNA approach to identify novel biomarkers making advantage of primary human hepatocyte (PHH) and rat in vivo liver toxicogenomics datasets. We systematically assessed the top 100 upregulated genes for the different stress pathways in both models and selected overlapping genes in both pathways. Additionally, protein expression and upregulation of these biomarkers of interest upon model compound exposure were assessed in iPSC-derived hepatocyte-like cells. This eventually led to a final set of 4-5 biomarkers for each cellular stress pathway. In the next phase we will use CRISPR/Cas9 technology to make fluorophore fusion reporter cell lines with these novel biomarkers. Subsequent differentiation into hepatocyte-like cells as well as other cell types in combination with high content imaging (HCI) will provide improved insight in the dynamics of the diverse types of cellular stress signalling and provide a critical innovative toolbox for chemical safety assessment.

POSTER 40 Methods to assess Developmental and Reproductive Toxicity (DART) with the zebrafish (Danio rerio)

Rebecca von Hellfeld[1], Katharina Brotzmann[1], Thomas Braunbeck[1]

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Abstract:

Originally designed for testing of the effluent and chemical toxicity, the zebrafish (Danio rerio) embryo toxicity test also proved to be a valuable tool for the assessment of developmental and reproductive toxicity (DART). Overall, depending on the endpoint(s) and the age of the fish, there is a multitude of test methods with the zebrafish covering various lethal and sub-lethal endpoints: e.g., the Fish Embryo Acute Toxicity Test (OECD TG 236), the Acute Fish Toxicity Test (OECD TG 203) and the Early-life Stage Toxicity Test (OECD TG210). In all of these tests, morphologically discernible (developmental) malformations, e.g., of eyes, heart, liver, head, fins and spine can be studied; likewise, endpoints can be studied at the level of genes and metabolites. Histological (paraffin) and frozen sections allow the examination on the cellular and organ levels; behavioural assays and an assessment of sexual development and reproductive fitness of the offspring extend the research opportunities beyond the parental generation. However, beyond the age of 120 h, such tests are regarded as animal experimentation. Since numerous studies document the comparability of observations in zebrafish and mammalian models, the zebrafish model overall represents a promising candidate to complement or even replace mammalian testing for toxicity and teratogenicity.

POSTER 41 Teratogenicity in mammals predicted by a non-mammal test system – the zebrafish (Danio rerio) embryo

Katharina Brotzmann[1], Rebecca von Hellfeld[1], Thomas Braunbeck[1]

[1]*University of Heidelberg, Germany*

Abstract:

The zebrafish (Danio rerio) has been used to study general toxicity of compounds in the Fish Embryo Acute Toxicity (FET) test (OECD TG 236), which looks at the non-protected developmental stage (the first 120 hours post fertilisation (hpf)) of the fish. The observed effects commonly consist of visible morphological or behavioural changes, as well as lethal endpoints. However, focus can be placed on specific effects, if these are found to closely resemble effects observed in other experiments or in patients. For example, this model could be utilised to investigate drug-induced liver injury (DILI) as well as for neurotoxicity screenings. The results strongly indicate liver, cardiac, neurological and teratogenic toxicity, depending on the compound in question. Similarities were found between these results and data obtained from mammal- as well as clinical studies. The test systems' ability to predict toxicity and teratogenicity, as well as being an emerging model for human diseases and drug discovery has already been proven in the past. Furthermore, approximately 70% of human genes are also present in the zebrafish genome and further investigations are ongoing.

POSTER 42 Comparison of toxicity patterns of 19 compounds across 21 organ-specific in vitro test methods

Alice Krebs[1], EU-ToxRisk cross systems group, Marcel Leist[1]

[1]*University of Konstanz, Germany*

Abstract:

The EU-ToxRisk cross-systems group, faced by the issue of multiple different laboratories contributing test results, aimed to introduce a unified and transparent procedure for data generation and annotation in a multi-stakeholder project. The test battery, contributed by 11 partner laboratories, comprises 23 human, organ-specific in-vitro test systems, often with several endpoints within each system. The test methods use 2D and 3D cultures, representing liver, brain, kidney and lung as organs. Moreover, some tests addressed potential developmental toxicity to immature differentiating cells and tissues, and yet other tests probed signalling pathways on the basis of reporter constructs in transgenic cells. A set of 19 model toxicants was compared in all tests.

We established a structured repository of method descriptions and test protocols and a universal format for deposition of experimental data and metadata according to the FAIR criteria. The test methods were quality evaluated and scored for their readiness for regulatory use. Summary data from testing comprised benchmark concentrations with measures of uncertainty. Characterization of test methods included their baseline variance and relative sensitivity. We found that in many cases functional endpoints were more sensitive than viability measures. Moreover, pronounced differences in sensitivity to toxicants were observed between test methods. Repeated-dose testing, transcriptomic studies and biokinetic measurements are currently conducted to improve the implementation of in vitro tests in risk assessment.

POSTER 43 (speed presentation) Metabolically improved stem cell derived hepatocyte-like cells as a promising tool to study toxicology and disease modelling

Tine Tricot[1], Manoj Kumar[1], Ruben Boon[1,2], Hendrik Jan Thibaut[3], Kayvan Abbasi[3], Johan Neyts[3], Catherine Verfaillie[1]

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[3]*Laboratory of Virology, Rega Institute, Katholieke Universiteit Leuven, Belgium*

Abstract:

In vitro models to study liver diseases and toxicology rely on the use of primary human hepatocytes (PHHs) and hepatoma cell lines such as HepG2 cells, which both have major limitations. Therefore, scientists are investigating alternative sources of hepatocytes such as pluripotent stem cells (hPSCs). Many different differentiation protocols exist to generate hepatocyte-like cells (HLCs) from hPSCs; however, such HLCs are not fully mature. The Verfaillie lab created (i) hPSC lines that inducibly overexpresses three or six liver-specific transcription factors (HC3x, HC6x) and (ii) optimised medium conditions for HC3x/HC6x-HLCs, which generate a more mature hepatocyte progeny. The genome engineering as well as the nutrient engineering only significantly improved cellular metabolism (HLCs are now gluconeogenic), but also induced global hepatocyte maturation and functionality (HLCs have drug metabolizing capabilities very similar to PHHs). To evaluate their ability to predict hepatotoxicity, HC3x-HLCs and HC6x-HLCs in optimised medium were tested with 13 training -compounds list from MIP-DILI consortium and compared to the performance of PHHs and HepG2 cells. The HC3x-HLCs and HC6x-HLCs in optimised medium were, unlike the HepG2, able to identify the hepatotoxicants to a similar extent as fresh PHHs. Furthermore, we demonstrated that the HC3x-HLCs can also be used as a model for liver diseases such as Hepatitis B virus (HBV). HC3x-HLCs could efficiently be infected with HBV, which was demonstrated by the expression of HBV core (HBc) and surface antigens (HBs) and by a clear release of HBs and HBe-antigens in the culture supernatants, which increased over time, indicating functional cccDNA formation. Moreover, high titres of infectious virus were detected in the culture supernatant that could infect secondary HepG2-NTCP cells. Additionally, the system can be used to test anti-HBV drugs. We are now planning to perform larger scale drug screens to identify novel and selective anti-HBV drugs to improve and expand the existing therapeutic regimens. Furthermore, we are investigating other diseases that can be successfully modelled with these improved HLCs.

POSTER 44 (speed presentation) Towards an ADME Competent 4-Organ-Chip

Beren Atac[1], Anja Wilmes[2], Liliana Monica Santos Capinha[2], Corinna Magauer[1], Julianna Kobolák[3], Frederic Y. Bois[4], Paul Jennings[2], András Dinnyés[3], Wolfgang Moritz[5], Uwe Marx[1]

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Abstract:

A PBPK modelled ADME-N 4-Organ-Chip (Chip4) with a downscale factor of 1:100.000 is utilized for establishing the co-culture of human IPSC derived proximal tubular-like cells (PTL) for a barrier, human primary liver spheroids for main metabolism, human primary intestinal barrier for absorption and human IPSC derived neuronal spheroids as a potential target organ. The initial experiments were dedicated to optimising culture conditions and the common medium formulation. In addition, a preliminary repeated HCBD exposure for 48 hours is conducted to understand the hurdles for a future repeated exposure of the compound for a longer duration.

POSTER 45 Cytokine analysis of human PCLS after drug treatment

Leroy Elenschneider[1], Tanja Hansen[1], C. Sandman[2], J. Fangmann[3], Karin Cederbrant[2]

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[2]*Research Institutes of Sweden (RISE), Sweden*
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Abstract:

Chemical induced liver injury remains a challenging issue because its underlying mechanisms are still not fully understood. Knowledge from drug-induced liver injury (DILI) points to a critical involvement of certain cytokines and we hypothesized that mechanisms of chemical induced liver injury might be similar. To test this hypothesis a panel of eight cytokines were analysed in supernatants of human precision-cut liver slices (PCLS) after 24 h treatment with two antidiabetic agents and with two organic solvents known to be associated with occupational liver disease. High levels of interleukin (IL)-33 release could be detected in all scenarios, but a dose-dependent effect could not be identified. IL-6 production decreased in a concentration-dependent manner in the samples treated with troglitazone (TGZ) and dimethylformamide (DMF), whereas the samples treated with dimethylacetamide (DMA) show the opposite effect. Tumour necrosis factor alpha (TNF-α) secretion was especially increased at high doses of TGZ, DMA and DMF but not in samples treated with rosiglitazone (RGZ). The additionally measured cytokines IL-18, IL-1b and IL-10 could not be detected in any occasion. IL-12 could be measured in all samples treated with antidiabetic agents but only partially in samples with organic solvents.

POSTER 46 Prediction of SNPs having potential e ects on the pharmacology and toxicology of chemicals

Alejandro Aguayo-Orozco[1], Bryan Dafniet[2], Søren Brunak[1], Jose Maria Gonzalez-Izarzugaza[1], Olivier Taboureau[1,2,3,*]

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[3] *INSERM UMR-S 973, France; *Presenting author*

Abstract:

The effect of individual genes due to a single mutation variation in response to a chemical compound is known as pharmacogenomics (PGx). Association of PGx and GWAS have been proved to be able to identify several genetic variants used to inform for toxic doses and chemical selection. In order to be able to prioritize SNPs, to be studied as possible effectors on patient cohorts having different pharmacological or toxicological reactions, we have developed a method, based on the secondary structure, amino acid properties and the 3D structure, when available, of proteins. We expect, this method to improve our understanding of different reactions to chemicals from different patient cohorts and different populations, as well as understanding the potential model of interactions involved in these effects. Examples are presented in the poster.

POSTER 47 (speed presentation) Transcriptomics-based statistical modelling of the inter-individual variability of drug-induced stress response activation

Marije Niemeijer[1], Frédéric Bois[2,*], Suzanna Huppelschoten[1], Audrey Baze[3], Celine Parmentier[3], Richard S. Paules[4], Lysiane Richert[3], Bob van de Water[1]

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Abstract:

The activation of adaptive stress response pathways is a key event in the development of chemical-induced liver injury. Statistically estimating the variance in activation of these stress signalling pathways between different individuals could reveal DILI susceptibilities and allow better mitigation strategies. Here, to determine the inter-individual variance of chemical-induced stress response activation, the transcriptome was systematically interrogated in a panel of 51 cryo-preserved primary human hepatocytes (PHHs) derived from different individuals using the BioSpyder TempO-Seq technology. Crucial stress response activation was examined for the oxidative stress pathway using diethyl maleate, the unfolded protein response using tunicamycin, DNA damage response using cisplatin and NFκB signalling using TNFα treatment in a broad concentration range for 8 and 24 hours. Individual concentration-gene activation modelling was performed to obtain benchmark concentration levels (BMC) and maximum fold-change (maxFC) for 50 top-activated genes for each individual. As a next step, the statistical distribution of BMDs and maxFCs of these top 50 genes for each subject and each chemical at each time point was modelled using a population mixed-effect framework. This allowed us to simulate the results of experiments with smaller (typical) or larger numbers of hepatocyte donors. From those simulations we extracted the predictive distribution of between-donors variability estimates. Our results showed that low numbers of donors systematically under-estimate BMDs variances by about a factor 2 and are imprecise in capturing the population variance of stress response activation. Based on this, we propose a set of safety factors to circumvent under-estimating between-subject variability to obtain improved predictions of DILI susceptibilities.

POSTER 48 (speed presentation) Linking p53 signalling to cell cycle progression using in silico modelling

Muriel Heldring[1], Lukas Wijaya[1], Sylvia Le Dévédec[1], Bob van de Water[1], Joost B. Beltman[1]

[1]*LACDR, Leiden University, the Netherlands*

Abstract:

Disruption of cellular homeostasis by chemical compounds can induce temporary or chronic liver injury or even complete failure. There is a vast array of proteins involved in homeostasis that controls the response to diverse types of stress. Among the most important is p53, primarily known for its function to maintain genomic stability and regulate cell cycle progression, senescence and apoptosis. To gain insight in the downstream effect of chemical-induced activation of the p53 pathway, we generated expression data of p53 and its downstream targets Mdm2, p21 and Btg2 using live-cell imaging of BAC-GFP HepG2 reporter cell lines exposed to various concentrations of cisplatin for 72 hours. Cell cycle progression was determined by measuring FUCCI expression of HepG2 cells in a similar imaging set-up. Here, we present an ODE model to provide a quantitative description of the observed protein expression dynamics and to link the expression patterns to cell cycle progression.

POSTER 49 (speed presentation) Bayesian network based qAOP modelling: application to mitochondrial complex I inhibition leading to neuronal cell death

Wang Gao[1], Ghislaine Gayraud[1], Frédéric Bois[2]

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[2]*Certara UK Limited, Simcyp Division, United Kingdom*

Abstract:

Motivation: While AOPs are useful for hazard assessment, they fall short of predicting risks because they do not consider exposure nor quantify dose-response relationships. Quantitative AOPs, with a structure imposed by an underlying AOP and a set of linked quantitative key-events relationships, should be useful for risk assessment. We propose to apply Bayesian networks (BN) modelling approach to AOP quantification and present in this poster the methods and applications of BN models to the AOP of mitochondrial complex I inhibition leading to neuronal cell death, which is investigated in vitro in case study 4 of EU-ToxRisk.

Result: We obtained a reasonable quantification of the mitotoxicity AOP in neurons for two reference chemicals: rotenone and deguelin. The behaviour of the qAOP obtained is interesting due to the branching of the AOP structure the level of mitochondrial respiration inhibition. We also evaluated the predictive power of the qAOP on a testing set constructed by eight other chemicals' data. From this validation exercise, we can conclude that the deguelin-based qAOP is reasonably predictive of the eight other chemicals tested and that the rotenone-based version systematically over-predicts toxicity.

POSTER 50 (speed presentation) Multistate models of valproic acid developmental toxicity in the zebrafish embryo

Ségolène Siméon[1], Rémy Beaudouin[1], Katharina Brotzmann[2], Frédéric Bois[3,*]

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[3]*Certara UK Limited, Simcyp Division, United Kingdom*
**Presenting author*

Abstract:

According to effect experimental assays, the OCDE guidelines recommend performing a separate dose-response analysis for each observation time. However, the events at a given time are partly conditioned by what occurred in the past. Splitting the data by time group affecting them with much reduced power. The standard analysis of such occurrence time data uses survival models. In addition, occurrences of malformations are not independent. For example, dead embryos do not develop subsequent heart malformation, so that, at lethal concentrations, heart malformation prevalence can decrease because embryos die before heart development. That is a competing risk problem. The generally recommended solution to complex survival problems is to use multi-state models. We develop multi-state survival models adapted to the OECD test to improve toxicity data analyses in the zebrafish embryo. 55 developmental malformations are experimentally observed during the five first days of life. We began by build a three-state model by grouping the effects in "Normal", "Hatched" and "Coagulated and Effects" states, to highlight the importance of modelling the hatching rate, with control embryos. Then, we built a more complex five-state model, sharing the "Effects" and "Coagulated" states. We estimated the transition rates from a state to the others using all the data and related them to toxicant concentration. Then, the numbers of malformations can be modelled and predicted as a function of time and dose.

POSTER 51 The EU-ToxRisk dissemination activities in figures

Giorgia Pallocca[1], and the EU-ToxRisk consortium

[1]*Steinbeis CAAT-Europe at the University of Konstanz, Germany*

Abstract:

From its start, the EU-ToxRisk project has been fostering a solid dissemination plan. To date, the consortium produced over 100 publications based on almost 950 deposited datasets from over 150 different new approach methodologies (NAMs). These methods have been extensively characterized and described, adhering to high-level quality parameters and have given rise to a publicly accessible methods database. Through the organization of several dedicated sessions at international conferences, workshops, and meetings, the project has opened fruitful discussion channels with regulators, industry stakeholders, and international toxicology programs. These knowledge exchanges are feeding into various tangible project outcomes, the EU-ToxRisk NAM-enhanced Read-Across (RAx) Advisory Document and the EU-ToxRisk Commercialization Platform, which will be made available for the broader toxicology community to move forward a reliable, animal-free hazard and risk assessment of chemicals.

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